Myopathy and hepatic lipidosis in weaned lambs due to vitamin E deficiency

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Abstract — A sheep flock experienced losses in weaned lambs from myopathy and hepatic lipidosis. Investigation revealed painful ambulation, illthrift, and unexpected death in lambs with normal selenium levels, deficient vitamin E levels, and elevated muscle and liver enzyme levels. Vitamin E deficiency should be considered when investigating myopathy and illthrift in lambs.

Résumé — Myopathie et lipidose hépatique causées par une déficience en vitamine E chez des agneaux sevrés. Des pertes se sont produites chez les agneaux sevrés d'un troupeau de moutons à la suite de myopathie et de lipidose hépatique. Une étude a révélé une démarche douloureuse, un mauvais état de chair et une mortalité inattendue chez des agneaux présentant un taux de sélénium normal, un taux de vitamine E bas et un taux d'enzymes musculaires et hépatiques élevé. Une déficience en vitamine E devrait être prise en considération lors d'enquêtes sur des agneaux présentant de la myopathie et un mauvais état de chair.

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The owner of a sheep flock contacted the flock veterinarian regarding a high morbidity and mortality rate in weaned lambs. The flock was comprised of 50 Suffolk ewes with 70 weaned lambs at risk, ranging in ages from 2 to 4 mo. The main presenting complaints were sudden deaths, as well as illthrift, weakness, and painful ambulation in many of the lambs. Diagnostic hypotheses included white muscle disease (WMD), ionophore toxicosis, pulpy kidney disease, copper toxicosis, and vitamin B12 deficiency. Necropsy of 5 of the affected lambs by the Animal Health Laboratory (AHL) (a division of the Laboratory Services Division) or the Veterinary Teaching Hospital (VTH) (Anatomic Diagnostic Pathology, Ontario Veterinary College), revealed significant changes in the skeletal muscle and liver. Gross findings included generalized atrophy of skeletal muscles, pallor and occasional white streaking of skeletal muscles without gross evidence of metastatic calcification, and pallor of the liver. Histological examination of skeletal muscle sections revealed acute to subacute myopathy, with swollen eosinophilic, hypercontracted and fragmenting myofibers, macrophage infiltration, and proliferation of satellite nuclei with minimal mineralization. Liver sections showed evidence of hepatopathy or hepatic lipidosis. Hepatocytes containing single clear cytoplasmic vacuoles and mild cholangiolar hyperplasia in portal tracts accompanied by macrophages containing grey-yellow pigment were commonly present in the parenchyma.

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Feed samples were negative for ionophores (lasalocid, monensin, and salinomycin). Samples submitted for liver copper analysis contained normal amounts of copper (normal range 100 to 350 μg/g liver dry weight) (1). A visit to the flock by the Ruminant Field Service, Ontario Veterinary College was arranged to further investigate the problem.

The flock had been established for many years; the only outside flock additions were periodic purchases of rams. Management had remained relatively unchanged. Productivity was not officially measured; in previous years, ewes commonly reared twins and triplets successfully and lambs were well grown by 3 mo of age. The breeding ewes were exposed to the rams in the fall without estrus synchronization and the current lambing occurred from February 16th to March 16th. The gestation and lactation ration included purchased mixed grass hay in small square bales, fed ad libitum, and free choice salt blocks containing trace minerals; 6 wk prior to lambing, purchased mixed grain was fed. Hay, grain, and commercial feeds were all stored in the barn or barn loft under clean and dry conditions. Lambs were reared indoors with free access to a creep area containing free choice commercial pelleted lamb grower (Lamb Grower 18; Land O Lakes, St. Paul, Minnesota, USA) and purchased mixed grass hay. The owner had purchased loose sheep mineral (Masterfeeds, London, Ontario), but it had only been offered recently to lambs and was withdrawn by the end of May. Water was obtained from a drilled well on-farm. Weaning occurred at approximately 60 d of age. Flock health procedures consisted of the administration of a prelambing anthelmintic drench (Ivomec; Merial Canada, Baie D'urf, Quebec), 200 μg/kg body weight, to all pregnant ewes; an injection of 0.25 mL of a commercial vitamin E selenium preparation (equivalent to 0.75 mg selenium and 34 IU vitamin E) (E-Sel; Citadel Animal Health Cambridge, Ontario) to all lambs at birth, and vaccination at 6 to 8 wk of age of all lambs with a multiway clostridial vaccine (Covexin 8; Schering-Plough Animal Health Division of Schering Canada, Pointe

Table 1. Summary of lamb mortality and diagnoses

ID	Died	Clinical comment	Disposition	Diagnosis
J13	Mar-01	weak at birth	died at 1 d of age	none
J22	Apr-02		died suddenly	suspected pulpy kidney[a]
J1	Apr-04		died suddenly	suspected pulpy kidney[a]
J6	Apr-07		died suddenly	none
J57	Apr-16		died suddenly	none
J15	May-01		died suddenly	none
J31	May-19		died suddenly	suspected pulpy kidney
J35	May-19	unable to rise[b]	euthanized	none
J18	May-21	weak, staggering	euthanized, necropsied	myopathy[c] hepatopathy
J19	May-21	weak, staggering	euthanized, necropsied	myopathy[c], hepatopathy
J16	Jun-03	weak, staggering	died, necropsied	myopathy[c], hepatic lipidosis
J17	Jun-03	weak, staggering	died, necropsied	myopathy[c], hepatic lipidosis
J24	Jun-03	weak, staggering	died, necropsied	myopathy[c], hepatic lipidosis
J7	Jun-05	unable to rise	euthanized	suspected myopathy
J23	Jun-15	unable to rise	euthanized, necropsied	myopathy[c], hepatic lipidosis
J14	Jun-16	unable to rise	euthanized, necropsied	myopathy, hepatic lipidosis

[a]Pulpy kidney disease was suspected by owner and/or flock veterinarian on the basis of an unexpected mortality in a lamb that otherwise had been considered healthy

[b]The owner reported that the lambs were either unable or unwilling to rise, or if able to stand, appeared to have difficulty ambulating. He was unsure if this difficulty in ambulating was due to weakness, pain, or neurological deficit. Veterinary clinical assessment ruled out neurological deficit

[c]Myopathy was primarily skeletal in location, with the exception of lamb J14, which also had cardiac myopathy

Claire, Quebec). Ewes were not routinely vaccinated. At birth, all navels were dipped in iodine and elastic rings were applied to tails and, in the case of ram lambs, to scrotums.

The suspected and known causes of lamb mortality are summarized in Table 1. Seven lambs had died prior to the flock veterinarian being contacted by the flock owner on May 19. By the end of the investigation, total neonatal mortality pre- and postweaning was 16 of 70 (22.3%) lambs (average lamb mortality for Ontario flocks is less than 7% [2]).

At the initial visit by the Ruminant Field Service on June 14, many of the lambs were thin and small for their age and breed. Expected average daily gains for this breed under this management, ranges from 400 to 550 g/d (2), which enables lambs to achieve market weight of 40 to 50 kg at less than 3 mo of age. In this flock, several of the lambs examined weighed less than 30 kg at more than 3 mo of age. On body condition scoring (BCS), these lambs were thin with several of the lambs scoring 2 or less out of a possible 5 (with the goal of 3.5 to 4 for lambs ready for market). Two of the most severely affected lambs (J14 and J23) were unable to rise without assistance and, when aided, were reluctant to stand for more than a few seconds. Both were euthanized and submitted to the VTH for postmortem examination, where it was found that both had skeletal muscle and hepatic findings similar to those of the 5 lambs necropsied previously. Lamb J14 also had grossly pale heart muscle with histological evidence of acute myocardial cell damage. Skeletal muscle lesions were classified as subacute with some acute lesions, indicating ongoing damage at the time of death or euthanasia.

Feces were submitted to the AHL for parasitologic analysis from a selection of affected and normal appearing lambs, as well as from 4 randomly selected ewes, 2 of which were still housed indoors, and 2 of which had been on pasture for 1 wk. Significant levels of coccidia or gastrointestinal parasites were not found on fecal examination. Serum and whole blood were also obtained from the same animals and submitted to the AHL for biochemical analyses. Results for the assays of serum vitamin E, creatine kinase (CK), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), and whole selenium (Se) by methods previously described (3–7) are presented in Table 2. All Se values for the lambs were within the normal range (0.12 to 0.5 mg Se/L) (1). One ewe was Se deficient. However, all lamb samples had vitamin E levels lower than 1.0 μg/mL (normal range 1 to 3 μg/mL, Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, Michigan, USA), and in 9 of the 12, the values were in the deficient range (< 0.5 μg/mL). The 2 housed ewes had deficient (< 1.0 μg/mL) vitamin E levels and the 2 ewes at pasture had normal levels (> 1.0 μg/mL, Diagnostic Center for Population and Animal Health). In the lambs tested, the CK values were elevated (normal 42 to 62 U/L[3], AHL) and compatible with ongoing muscle damage. In some of the animals, AST, γ-GT, or both, were elevated, suggestive of ongoing liver damage.

Based on these findings, the ration was investigated further to determine if the diet was deficient in vitamin E. Feed samples were analyzed by the AHL to determine Se and vitamin E levels. The oat barley grain mixture contained very low levels of Se (< 0.05 μg/g); the generally recommended feed level is a minimum of 0.1 μg/g dry matter (DM) Se (8). The hay contained 0.27 μg/g DM Se, which is considered adequate, and the commercial 18% lamb grower and sheep mineral mix contained values approximating label claims (3.3 and 43 μg/g DM, respectively). Analysis of the hay found vitamin E levels at 11.6 μg/g DM, which is lower than ranges given for sun-cured mixed hays (20 to 100 μg/g DM) (9). The oat barley mix was found to contain only 5.89 μg/g DM vitamin E, approximately 25% of the level listed for these grains (9). Since these feeds had been harvested and stored from the previous year, loss of a portion of the naturally occurring vitamin E was not unexpected. The commercial lamb grower was listed as containing 22 μg/g minimum of vitamin E and was found to contain 21.1 μg/g. The recommended daily supplementation of vitamin E for early weaned lambs with rapid growth

Table 2. Selected analytical results for clinically normal and abnormal lambs and ewes in order of increasing serum vitamin E level

ID	Clinical comment	VitE (μg/mL)	CK (U/L)	AST (U/L)	γ-GT (U/L)	Se (mg/L)	History
J14	small, weak, unable to rise	0.17	32700	6130	50	0.54	euthanized
J50	BCS < 2, small	0.2	1269	386	122	0.3	
J2	BCS = 2, small	0.29	1746	408	85	0.51	twin of affected lamb
J58	BCS = 1.5, small	0.36	340	127	49	0.66	
J38	BCS = 3, small	0.37	210	101	58	1.1	
J8	BCS = 3.5, well grown	0.41	126	199	97	0.55	
J43	BCS = 3.5, well grown	0.41	172	85	66	0.28	
J56	BCS = 2.5	0.43	319	118	67	0.92	
J36	BCS = 2, small	0.48	142	85	61	0.27	twin of affected lamb
J70	BCS = 2, small	0.56	181	101	69	0.36	
J23	small, weak, unable to rise	0.58	832	311	43	0.45	euthanized
J42	BCS = 2, small	0.69	145	114	60	0.34	
968 (ewe)		0.79	118	87	67	0.59	lost lambs
738 (ewe)	BCS 4, healthy	0.81	102	105	60	0.71	did not lamb
J59	BCS 3.5, well grown	0.96	143	91	50	0.3	
829 (ewe)		1.66	82	187	121	0.11	on pasture
610 (ewe)	BCS = 2.5, thin	2.03	93	97	49	0.2	on pasture, lost lambs J18 & 19

Reference range for vitamin E: deficient < 0.5 μg/mL; adequate 1.0 to 3.0 μg/mL for juvenile lambs (Michigan State University)
BCS — body condition score; range 1 to 5; CK — Creatine kinase (reference range, 42 to 62 U/L) (Animal Health Laboratories, University of Guelph); AST — Aspartate aminotransferase (reference range, 90 to 260 U/L) (Animal Health Laboratories, University of Guelph); γGT — Gamma-glutamyl transferase (reference range, 25 to 60 U/L) (Animal Health Laboratories, University of Guelph); Se — selenium (reference range, 0.12 to 0.5 mg/L) (Animal Health Laboratories, University of Guelph)

potential should be 21 to 25 IU per animal (9). The expected DM intake for 20 to 40 kg lambs ranges from 1.2 to 1.5 kg DM/d (9). At these intakes, it is likely that the lambs were consuming adequate grower and hay to meet NRC requirements for vitamin E. The shelf life of vitamin E used in the manufacture of commercial feeds is considered to be more than 6 mo. This commercial feed had been purchased within the previous 3 mo from a local supplier. Given these results, it was reasonable to expect that supplementation of the lambs with the commercial lamb grower, despite the inadequate levels of vitamin E in the hay, should have met the lambs' dietary needs.

The prevalence of clinical vitamin E deficiency, without concurrent Se deficiency, in lambs is not known. Lipid soluble vitamin E is part of the antioxidant system that protects those cellular components high in lipids (such as the cellular membrane, mitochondria, endoplasmic reticulum, and plasma membranes) from lipoperoxidation by decreasing hydroperoxide formation (10). Selenium, which is a necessary component of glutathione peroxidase (GSH-PX), also acts to reduce oxidative damage by destroying peroxides in the body's more aqueous environment. Diets high in vitamin E, but deficient in Se, may still result in the development of WMD in lambs, since the nonmembrane proteins are not protected by the GSH-PX system and so are at risk of oxidative damage. Conversely, diets deficient in vitamin E, but adequate in Se, also may put at risk some subcellular components that are not protected by the GSH-PX system (10). Vitamin E deficiency usually occurs in animals that are fed poor quality hay with no other source of vitamin E or diets that are high in polyunsaturated fatty acids. Unaccustomed exercise will worsen the myopathy. These risk factors were not present in this flock.

Typical WMD lesions in lambs include marked metastatic calcification of muscle fibers. The muscle lesions in this case were more subtle than those usually seen with WMD and on gross examination could be further differentiated from those of WMD because of the lack of metastatic calcification in the muscle lesions. The lambs in this investigation also had evidence of liver damage.

Few cases of naturally occurring vitamin E deficiency without concurrent Se deficiency have been reported in lambs. Experimental vitamin E myopathy has been induced in lambs by adding cod-liver oil to the milk replacer (11). The affected lambs grew more slowly, had low vitamin E serum levels, and elevated CK levels. Two of the lambs also developed generalized myopathy characterized by pale musculature and swollen fibers in the semitendinosus muscles and myocardium. Mild hepatosis was also seen. This is very similar to what was found in the flock under investigation in this report.

Naturally occurring vitamin E myopathy in nursing lambs was reported in 2 farm flocks in Idaho (12). In both flocks, lambs were found to have normal serum Se status but low serum vitamin E levels. Neither flock was receiving supplemental vitamin E in the lamb or ewe ration. Clinically, the myopathy appeared to be similar to that of WMD, but on necropsy, it was differentiated by the absence of metastatic calcification. Clinically, both flocks responded slowly to vitamin E supplementation. In another report, an outbreak of subcapsular liver rupture was seen in a group of lambs suspected of suffering from vitamin E deficiency (13). Eight of 22 lambs died within the first 3 wk of life. Necropsy revealed abdominal hemorrhage and a friable liver. However, hepatocytes were normal. Vitamin E serum levels in the flock were found to be in the low to deficient range and Se serum levels were normal. The authors speculated that this disease is similar to hepatosis dietetica in swine due to vitamin E deficiency. Both these naturally occurring

cases (12,13) had findings similar to those in this case, although subcapsular liver rupture (13) was not seen; but it occurred in younger lambs, which may have contributed to the different presentation.

In this investigation, upon receiving the diagnosis of vitamin E deficiency, the owner administered a commercial preparation of vitamin E and Se, IM, to all remaining lambs. No more mortality occurred. Because of myopathy, poor growth, and low BCS in most of affected lambs, the owner, who operated a freezer trade business, elected to send the lambs to salvage slaughter rather than to finish them to suitable market weights, because of the risk of selling poor quality carcasses to his valued customers.

Although vitamin E deficiency without concurrent Se deficiency is not often reported in lambs, it is possible that it occurs more commonly than it is diagnosed. A recent survey of vitamin E levels in lamb and sheep livers in Ontario revealed that 90% of livers from market lamb had values that were below normal (14). A recent survey of submissions to UK veterinary investigation centers showed that the proportion of serum samples in April that fell into the vitamin E deficient range was from 40% to 80% (15). It may be that disease due to vitamin E deficiency alone is misdiagnosed as WMD or as sudden death due to misadventure (ruptured livers from trauma). Nursing lambs must obtain most of their vitamin E intake from the dam's colostrum and milk. Supplementation of vitamin E during the winter months, when it is not available from stored forages, must occur daily to ensure optimum health of the newborn and nursing lamb. Supplementation to growing lambs, when fresh pasture is not available, must also occur on a daily basis. It may be that NRC-listed vitamin E requirements are not adequate for fast growing lambs, necessitating that higher levels should be routinely supplemented. This investigation demonstrates the importance of examining vitamin E status when confronted with illthrift, skeletal or cardiac myopathy, or hepatopathy in growing lambs, even when they are receiving vitamin E supplementation. More work needs to be done on the effect of varying levels of vitamin E supplementation on the health of nursing and grower lamb, in the face of normal Se status. CVJ

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